EXPERIMENTAL INVESTIGATION OF VARIOUS FATS AS TO THEIR NUTRITIONAL VALUE

by

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I. INTRODUCTION

There are various kinds of lipids, such as simple lipid, phospholipid, glycolipid, sterol, etc. When the simple lipid is hydrolyzed with the aid of acid, alkali, or enzyme, glycerin and several kinds of fatty acids are produced. Esters of glycerin are called glycerides, and when all three hydroxyl groups of glycerin are esterified triglyceride is formed. Natural simple lipid usually takes the form of a triglyceride, and we can assume that the fat in our daily food takes for the most part this triglyceride form. Recently importance has been laid upon the nutritional value of the fat in connection with the clarification of the metabolic process. The quantitative study of necessary fat intake per day has become an important subject in the field of nutrition. DEUEL advocated the theory that one third of daily caloric intake should be from fats. Oral food intake for surgical patients, however, is sometimes prohibited entirely or limited to some extent. In such a case a nutriment has to be supplied by the parenteral method. This leads to the problem of the parenteral administration of fat. There are two things in this which attract our attention; one, what kind of fat to supply and two, how to supply it through parenteral administration. The first thing we think of as a means of parenteral administration of fat is an attempt to dissolve it in water. So far the experiment of dissolving fat in water has been carried out with the idea that if fat could be dissolved in water it might easily be transfered into blood, tissue fluid, and even tissue itself. But no noticeable results have been found as to the nutritive effect of the dissolved fat, probably because of the imperfect method of dissolving fat in water.

The author aimed at dissolving fat in water from a new stand point, and investigated whether or not a water soluble synthetic fat solution is effective as a parenteral fluid. The author has reevaluated the effect of the fat which we have been administering intravenously in the form of fat emulsion, and has further studied what kind of fat is most effective for the human body from the biochemical view-point.

I have synthesized the water soluble fatty acid salts in the purest form and investigated their toxicity. In order to make higher fatty acids, having the essential value of fat, water soluble, the non-toxic hydroxyl group was conjugated. Theoretically this is assumed to have a surface activity, and hemolysis was used as a criterion for its investigation. In order to lessen the hemolysis hydroxylated acids which are seen in the metabolic process in the body were synthesized and were further conjugated with the hydroxyl group. Their toxicity and nutritive value were investigated.

Furthermore, a quantitative study of various kinds of fatty acids shifting into various visceral organs was made, using the method of fatty acid paper chromatography.

II. MATERIALS AND METHODS

A) MATERIALS

1) Synthetic water soluble fats

i) Water soluble fatty acid salts

These were obtained in a refined state, by a different method from OMURA's. Caproic acid, caprylic acid, and capric acid were redistilled, dissolved in ethanol, heated to approximately 40°C, and were stirred as sodium hydroxide alcohol was added to neutralize them, using phenolphthalein as an indicator. At the point of neutralization, alcohol was evaporated in a warm water bath, and they were further dried under reduced pressure. When their sodium salts were dissolved in water and an excessive amount of pure calcium chloride was added, their calcium salts were obtained as precipitates. These were filtrated and were washed several times with distilled water.

ii) Sucrose monostearate

Synthesized by the method of OSIPOW, L. et al.

iii) Hydroxylated acids

Dihydroxystearic acid and tetrahydroxystearic acid (sativic acid) were synthesized as synthetic hydroxylated acids; and trihydroxypalmitic acid (aleuritic acid) was separated from shellac as a natural hydroxylated acid. Dihydroxystearic acid was obtained by oxidation of oleic acid using HILDITSCH's method. Tetrahydroxystearic acid was obtained by oxidation of linoleic acid using HAZURA's method. Aleuritic acid was separated from Indian shellac using GIDVANI's method.

iv) Glucose ester of dihydroxystearic acid

This was obtained as a result of the reaction of the methyl ester of dihydroxystearic acid and glucose under heat and reduced pressure using dimethylformamide as a solvent, and K_2CO_3 as a catalyst. It was further refined as it was passed through ion exchange resin equipment.

2) Fat emulsion

Three kinds of emulsion (15~20% sesame oil emulsion, 15% cod liver oil emulsion, and 15~20% synthetic simple triglyceride [C_8 C_9 C_{10}] emulsion) were made in our laboratory and 3.3 cc per kg was administered intravenously.

3) Experimental animals

The following animals were used for the experiment; young mice weighing less than 20 g for investigation of toxicity of water soluble fat; full grown male rats weighing approximately 200 g for investigation of nutritional value; full grown cats representing carnivorous animals with great ability to dispose of fat, rabbits representing herbivorous animals with poor ability to dispose of fat, and rats representing omnivorous animals were used in the investigation of fat metabolism after administration of the emulsion.

B) METHODS

1) Investigation of hemolysis

Since toxicity due to surface activity usually causes hemolysis, this was investigated by OGATA's method as an index of toxicity. The degrees of hemolysis were as follows: O:none, ?:faint trace, 1':trace, 1:distinct, 2':marked, 2:very marked, 3':almost complete, 3:complete.

2) Experimental investigation of acute toxicity

Observations were made on each group of five young mice weighing approximately 15 g which received a certain amount of the solution (sucrose monostearate and glucose ester of dihydroxystearic acid) intravenously in their tails.

3) Experimental investigation of chronic toxicity

A certain amount of the solution was given to each group of young mice weighing 15 g～20 g for one month by daily subcutaneous injections or by oral feeding.

4) Evaluation of nutritional effect

In order to evaluate the nutritional effect of hydroxylated acids and esters of hydroxyl groups, experiments were done with undernourished animals. Healthy male rats weighing approximately 200 g were fed 1/3 of the minimal calories needed to maintain body weight in a diet of Japanese sweet potatoes (Satsumaimo) containing mainly carbohydrate, of hydroxylated acids given orally and of Ebios (vitamin mixture); in addition the glucose ester of dihydroxystearic acid was given subcutaneously.

5) Extraction and separation of the fatty acids

Each organ was ground in a basin with sea sand and was heated for sixty minutes at about 60°C with BLOOR's solution and petroleum ether; the filtrate was concentrated and saponification was carried on for three hours by adding 1-N KOH alcohol. After the removal of the non-saponifiable matter, it was made acidic with 3-N HCl and was accumulated in ether layer, and was washed with water and the ether was evaporated.

6) Determination of neutralization value

The neutralization value was determined by adding drops of N/20 KOH alcohol, using phenolphthalein as the indicator.

7) Determination of iodine value

The iodine value was determined by WIIS's method.

8) Paper chromatography

Paper chromatography was done by NODA-HIRAYAMA's method, i. e. the development was made as the *p*-bromophenacylester 2,4-dinitrophenylhydrazones of fatty acids.

i) Filter paper

"Toyo" filter paper No. 2 was used. A starting line was penciled 3 cm above the upper border of the surface of the solvent.

ii) Solvent systems

As the moving solvent, methanol-glacial acetic acid-petroleum hydrocarbon (10 : 2 : 1. 2 by volume) was used, and petroleum hydrocarbon was used as the stationary solvent.

iii) Procedure

The paper was spotted with samples and was uniformly sprayed with the stationary solvent, petroleum hydrocarbon. Then the chromatogram was developed with the moving solvent by the ascending technique at 30°C. The development time required for a satisfactory separation was 5～6 hours by this method.

iv) Spot identifications

The spot identifications are as follows: The number represents the carbon atoms,

4 ; butyric acid, 6 ; caproic acid,
8 ; caprylic acid, 10 ; capric acid,
12 ; lauric acid, 14 ; myristic acid,
16 ; palmitic acid, 18 ; stearic acid,
20 ; arachidic acid, OL. ; oleic acid,
LE. ; linoleic acid, LN. ; linolenic acid,
DO. ; docosenoic acid (cetoleic acid),
EI. ; eicosenoic acid,
HU. ; highly unsaturated acids,
S. ; Standard, i. e. a mixture of the derivatives of all the even-numbered saturated acids from C_4 to C_{20}.

III. RESULTS

1) Sodium salts of caproic acid, caprylic acid, and capric acid are completely water soluble, but have strong surface activity causing marked hemolysis (Table 1). Their sodium salts show the strength of surface activity measured by the foam test, in the following order: C_{10}, C_8, C_6. The calcium salt of caproic acid is water soluble up to 2～2.5%, and causes distinct hemolysis. The calcium salt of caprylic acid is partially water soluble (less than 1%), and the calcium salt of

Table 1. Hemolysis of fatty acid esters

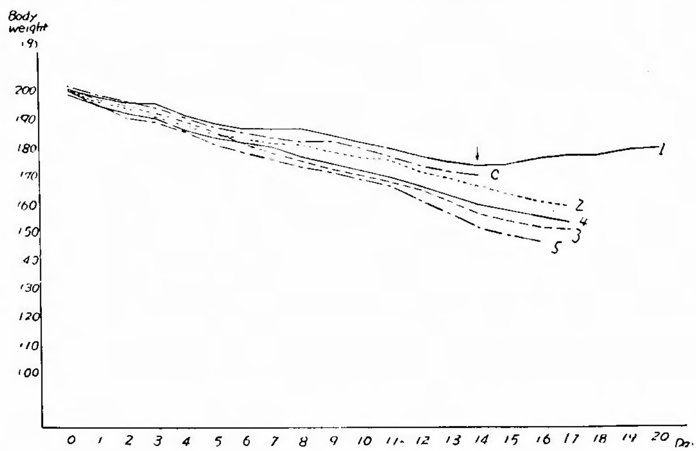
	10%	5%	2.5%	1.25%	0.6%	contr.
Na. capronate	1	1	3	3	3	0
Na. caprylate	3	1	1	3	3	0
Na. caprate	3	3	3	3	3	0
Ca. capronate			1′	3	3	0
Ca. caprylate	water insoluble					
Ca. caprate	water insoluble					
Sucrose monostearate	3	3	3	3	3	0
d. s. g. e.	?	0	?	1′	1′	0

capric acid is not water soluble (Table 1).

2) Sucrose monostearate synthesized as a sucrose ester of higher fatty acids is water soluble but causes strong hemolysis, and when this is given intravenously into tails of mice they all die immediately (Table 1).

3) Dihydroxystearic acid, tetrahydroxystearic acid (sativic acid), and trihydroxypalmitic acid (aleuritic acid), which are synthesized or separated as hydroxylated acids, are all nontoxic but are very poor in nutritional value and cannot prevent weight loss. Undernourished rats like to eat hydroxylated acids mixed with Ebios, but unlike the case of sesame oil (emulsion) feeding, they show a marked weight loss which cannot be prevented even with double dose feeding (Fig. 1).

Fig. 1 nutritional effect of hydroxylated acids



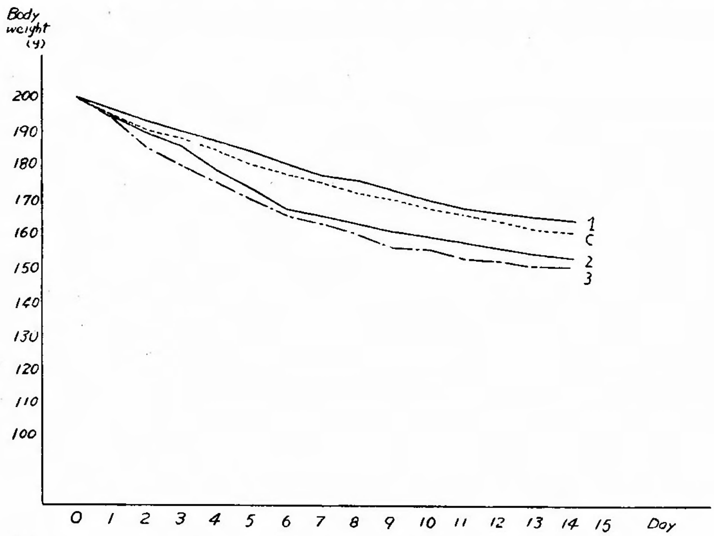
c : control sweet potato 10g + ebios 1g
group 1 sweet potato 10g + ebios 1g + sesame oil 1g
group 2 sweet potato 10g + ebios 1g + dihydroxystearic acid 1g
group 3 sweet potato 10g + ebios 1g + sativic acid 1g
group 4 sweet potato 10g + ebios 1g + aleuritic acid 1g
group 5 aleuritic acid 3g + glucose 1.8g (mol ratio)

4) The glucose ester of dihydroxystearic acid (d. s. g. e.) which is synthesized as a hydroxyl ester of hydroxylated acid causes less hemolysis and is not toxic (Table 1), but still its nutritional value is poor and there is a marked weight loss in comparison with the control group (Fig. 2).

5) Sesame oil and cod liver oil were analysed by paper chromatography. Sesame oil was found to contain essential fatty acids which have important nutritional value for the body, such as linoleic and linolenic acid, and also higher fatty acids such as myristic acid, palmitic acid, stearic acid, oleic acid etc. Cod liver oil was found to have in addition highly unsaturated fatty acids (having more than 4 double bonds), docosenoic acid, eicosenoic acid, etc., in large amounts (Fig. 3).

6) Liver, kidney, and heart muscle of cats in the postabsorptive state contained only essential fatty acids and higher fatty acids, and no lower fatty acids. This was also true in various organs of rabbits. A characteristic finding

Fig. 2 nutritional effect of the glucose ester of dihydroxystearic acid



c : control sweet potato 10g only
group 1. sweet potato 10g + 5% glucose 8cc + vitamin mixture
group 2. sweet potato 10g + 5% d. s. g. e. 8cc + vitamin mixture
group 3. sweet potato 10g + 5% d. s. g. e. 8cc

in cats which was not seen in any other experimental animals was the distinct demonstration of lauric acid in the kidney (Fig. 4).

7) Tricaproin, tricaprylin, and tricaprin which are synthetic simple triglycerides of lower fatty acids were mixed in the same proportion, made into an emulsion, and infused into cats intravenously. Three hours later the cats were sacrificed by bleeding and the fatty acid content of their various organs was investigated. We have found that these lower fatty acids had shifted only to the liver, and not to any other organs at all (Fig. 5).

8) Cod liver oil emulsion was infused into cats intravenously and three hours later they were sacrificed by bleeding. Their various organs were investigated for the fatty acid content as in the experiment mentioned above. We have found that highly unsaturated fatty acids, docosenoic acid, and eicosenoic acid had largely shifted to the liver (Fig. 6).

9) Sesame oil emulsion was infused into cats intravenously and three hours later they were sacrificed by bleeding. Their various organs were found to contain an increased amount of fatty acids without any change in their composition (Fig. 7).

10) When cod liver oil emulsion was repeatedly infused into rabbits intravenously at a rate of 3.3 cc of a 15% emulsion per kg per day for a period of approximately 7∼8 weeks, highly unsaturated fatty acids, docosenoic acid, and eicosenoic acid became markedly increased in the liver (Fig. 10).

11) Sesame oil emulsion was repeatedly infused into rabbits intravenously at a rate of 10 cc of a 20% emulsion per kg per day (three times larger than

the usual dose) for a period of approximately 20 weeks, and still the rabbits did not show any evidence of malnutrition. In comparison with the normal one (Fig. 8) the paper chromatogram showed an increase of linoleic acid which is an essential fatty acid, and no change in the fatty acid compositon of various organs (Fig. 9).

12) When the feedings of the cats were ceased abruptly, no marked change was observed in three days (Fig. 11), but after about 12 days weight loss was noted and fat tissue including that in subcutaneous tissue and omentum disappeared. At this stage when fatty acids in the various organs were analysed by paper chromatography, no decrease was found in the essential fatty acids such as linolenic acid and linoleic acid and a marked decrease was found only in the higher fatty acids (Fig. 12). The same finding was seen when rabbits were used (Fig. 13).

13) When the rats were fed a non-fat diet for a long period of time the higher fatty acids did not show any decrease since they are synthesized in the body, but the essential fatty acids which are not synthesized in the body showed a definite decrease (Fig. 14).

14) When sesame oil was heated with hot air for a long time, the peroxide value increased and the iodine value decreased. When the sesame oil was analysed by paper chromatography the saturated fatty acids showed no change, and essential fatty acids having double bonds, showed a definite gradual decrease. At the time of this reaction the amount of 2,4-dinitrophenylhydrazones produced was small and a black viscous precipitate was seen which was insoluble in ether. When eatable sesame oil was heated (over 200° C); it became gradually brown tinged, increased in viscosity and polymerization of fatty oils definitely took place. In this case the essential fatty acids in the sesame oil showed a definite decrease (Fig. 16).

IV. DISCUSSION

Generally speaking, what we usually call fat is triglyceride which is an ester of fatty acid and glycerine. There are various kinds of fatty acids, from lower to higher fatty acids which compose this triglyceride. Natural saturated fatty acids are said to have no side chains and have even numbers of carbon atoms. Lower fatty acids containing fewer than 10 carbon atoms are partially water soluble and are called soluble fatty acids. Acetic acid and butyric acid having small numbers of carbon atoms are completely water soluble, and as the number of carbon atoms increases the water solubility decreases, becoming completely water in-soluble above C_{10}. Water in-soluble substances like PAS or penicillin are well known to become water soluble when they take the form of sodium salt or potassium salt. Fatty acids are no exception and their sodium and potassium salts become water soluble. The initial attempts of parenteral administration of water soluble fat were all done in this form of alkaline salts. The water soluble fatty acid salts which OMURA reported in 1955 are also sodium, potassium, and calcium salts of water soluble lower fatty acids. Since the sodium salts of higher fatty acids above C_{12} are what we call soap, they cannot be used for parenteral administration because of their alkaline effect due to hydrolysis and a strong hemolysis due to surface activity which will be mentioned later. OMURA stated that he used lower fatty acids containing fewer carbon atoms than capric acid, and their sodium and potassium salts formed neutral salts without causing hydrolysis, therefore they could be used for parenteral administration. Thus OMURA made water soluble fatty acid salt solutions for parenteral use mixing the sodium, potassium, and calcium salts of these lower fatty acids in the same ratio as DARROW's solution.

I have synthesized sodium salts and calcium salts of C_6, C_8, and C_{10} in the purest form, and found that they caused a marked hemolysis and are not suitable for clinical use. Moreover, although the higher fatty acids possess high calories and play an important role as a variable element, lower fatty acids of less than C_{10} have a low caloric value ($2.6 \sim 3.7$ Cal. per g) in spite of the fact that they are immediately oxidized in the body as SCHOENHEIMER & RITTENBERG pointed out. In addition to this, as lower fatty acids are shifted solely to the liver which will be mentioned later, one may assume a heavy burden and a marked ketone body production in the liver. Nowadays it is not an exaggeration to say that the nutritional value of fat can be determined by the presence of the essential fatty acids which are indispensable for the composition and function of tissue cells. Therefore one cannot expect a nutritional effect which is specific of fat by administration of only lower fatty acids lacking the essential fatty acids. The lower fatty acids seem to be far inferior from a theoretical view point to sesame oil emulsion which contains enough essential fatty acids and also a high caloric value. Therefore, higher fatty acids must be made water soluble in the practical application of fat for parenteral use.

It is a widely practiced method to add sodium as a hydrophilic group in order to make water in-soluble substance water soluble. In the case of fatty acids, however, sodium salts of higher fatty acids which are weak acids become alkaline because of hydrolysis, except for the lower fatty acids which are strong acids. Since in addition to the sodium salt the hydroxyl group is another usable non toxic hydrophilic group, glycerin, glucose, and sucrose, having hydroxyl groups, are all non toxic and completely water soluble. Therefore hydroxyl esters of the fatty acids may prevent the alkaline reaction due to hydrolysis.

But it is known that when hydrophobic and hydrophilic groups are combined, surface active agents are produced. When a compound containing a lipophilic (non-polar) group and a hydrophilic (polar) group in one molecule is added to a water and oil mixture, combinations occur between the lipophilic group and oil, and between the hydrophilic group and water, which serve as bridges, lowering the surface tension of the oil and thus emulsifying the oil in water. The side effects of surface activity in the body are manifested as hemolysis and necrosis of tissue, even though their mode of action has not yet been clarified. Approximately 20% of sucrose monostearate which is a hydrophilic ester of stearic acid can be dissolved in hot water, but it causes marked hemolysis. Among sucrose esters, sucrose monostearate has the least surface activity, therefore, we may consider that the others have less value.

Thus the non•polar-polar compounds which newly synthesized by the combination of hydrophobic fatty acids and hydrophilic groups have surface activity which brings on side effects like hemolysis. It is supposed that in order to get rid of the surface activity if OH-radical is introduced into hydrophobic groups and this is combined with hydrophilic groups, solubility will increase while the surface activity will decrease. When fatty acid is burned in the body it is said to go through the stage of hydroxylated acids due to β-oxydation.

Therefore, dihydroxystearic acid and tetrahydroxystearic acid were synthesized as synthetic hydroxylated acids having a hydroxyl group introduced into the fatty acid. As a natural hydroxylated acid, trihydroxypalmitic acid that is aleuritic acid was separated from shellac. The toxicity and nutritional effect of these fatty acids were investigated using rats kept in an undernourished condition. As shown in Fig. 1, the result was contrary to what had been expected, and the hydroxylated acids were found to have no toxicity but no nutritional effect either. According to the theoretical calculation (LIPMANN's method, 1946), dihydroxystearic acid, tetrahydroxystearic acid, and aleuritic acid have 8.3 Cal., 7.3 Cal., and 7.4 Cal. respectively. It seems, however, that these fatty acids do not enter into a normal metabolic process in the body.

When a hydrophilic group is combined with a hydrophobic higher fatty acid such as stearic acid, palmitic acid, oleic acid, etc., in order to make them water soluble, the newly synthesized non•polar-polar compounds have surface activity and cause hymolysis. It is supposed that the hydrophilic group has to be combined after the introduction of a hydroxyl group into the (hydrophobic) fatty acids themselves, in order to lessen the surface activity. It has been also proved that the hydroxylated acids with hydroxyl groups have no nutritional effect and are not valuable in vivo, though they are not toxic.

Further we chose dihydroxystearic acid which is easily obtainable as a hydroxylated acid, to which glucose was combined as a hydrophilic group, synthesizing glucose ester of dihydroxystearic acid. Its toxicity and nutritional effect were investigated, and as shown in Fig. 2, it was proved that the toxicity as indicated by hemolysis was low but the nutritional value was much less than that of the control group.

Thus at the present stage it has been proved that none of the water soluble fats made by the theoretically possible methods are valuable for parenteral use. This leads to the conclusion that the parenteral administration of fats should be done intravenously in as emulsions as we have been doing.

The next question is what kind of fat is the most appropriate for the human body by this method of administration.

Our colleagues, S. ASADA and K. IZUKURA have used sesame oil emulsion, cod liver oil emulsion, and synthetic triolein emulsion for oral and intravenous administration in experimental animals, and have carried out a histochemical study of various organs. They found that the injected fat corpuscles left the blood stream within 30 minutes and were phagocytized by the alveolar phagocytes of the lung, KUPFFER's stellate cells of the liver and reticuloendothelial cells of the spleen, and that, they underwent a change in these cells from glyceride to phospholipid, the latter being shifted to the parenchymal cells of the liver or the extrahepatic tissues. Further quantitative study was done on the phospholipid shifted to the parenchymal cells of the liver and it was found that the amount of phospholipids differed according to the kinds of fatty acids. When cod liver oil, containing a large amount of highly unsaturated fatty acids, docosenoic acid, and eicosenoic acid, or butter, containing lower fatty acids, were given orally or intravenously as an emulsion, much larger amounts of phospholipids were demonstrated in the parenchymal cells of the liver, in comparison with the cases in which sesame oil emulsion was used containing nothing but higher saturated fatty acids, oleic acid and the essential fatty acids. This fact was confirmed by our colleagues H. SHIROTANI, and S. FUJINO's experiment using radioactive P^{32}, and our colleague T. KUYAMA found the same results as he studied the phospholipid content of various organs biochemically. According to the findings of our colleagues mentioned above, highly unsaturated fatty acids, lower fatty acids, eicosenoic acid, docosenoic acid etc. are shifted only to the parenchymal cells of the liver; and higher saturated fatty acids, oleic acid, and the essential fatty acids are shifted not only to the parenchymal cells of the liver but also to extrahepatic tissues where they will be disposed further.

The author, using paper chromatography of fatty acids, reaffirmed the fact that the amount of fatty acids in various organs differs according to the kind of the fatty acids, which our colleagues have indirectly proved using histochemical and biochemical methods.

Cats, representing carnivorous animals having a high capacity for the disposition of fat, were used. The synthesized simple glycerides of lower fatty acids, tricaproin, tricaprylin, and tricaprin were mixed in the same proportion and emulsified, and were administered intravenously. Histochemically it was proved that the amount of phospholipids shifted to the parenchymal cells of the liver reached the maximum three hours after injection. At this stage the experimental animals were sacrificed by bleeding, and the liver, kidney and heart being important parenchymal organs for the disposition of fat were removed, and the contained fatty acids were separated and analysed by paper chromatography. Caproic acid, caprylic acid, and capric acid in the group of lower fatty acids were shifted only to the liver and nowhere else (Fig. 5). When cod liver oil emulsion was administered, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. contained in the cod liver oil in a large amount, also seemed to be concentrated in the liver (Fig. 6). These fatty acids, however, were sometimes demonstrated in the extrahepatic tissues such as heart muscle in the postabsorptive state, therefore one had to assume that a portion of these fatty acids were shifted to the extrahepatic tissues, though the major portion were shifted to the liver. After the administration of sesame oil emulsion, the amount of fatty acids contained in various organs showed a marked increase, with almost no change in the composition of the fatty acids (Fig. 7).

In other words, lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. are shifted only to the parenchymal cells of the liver in the form of phospholipids and undergo further metabolism, and they are the ones which undergo, for the most part, socalled indirect oxidation. Whereas higher saturated fatty acids, oleic acid, the essential fatty acids etc. are shifted in the form of phospholipids not only to the parenchymal cells of the liver but also directly to the extrahepatic tissues and undergo further metabolism, and they are the ones which, for the most part, undergo socalled direct oxidation.

This was reevaluated using rabbits; cod liver oil emulsion was repeatedly administered intravenously for a period of 7～8 weeks and the fatty acids contained in the various organs of the above mentioned animals were analysed by paper chromatography. The highly unsaturated fatty acids, as well as docosenoic acid and eicosenoic acid having only one unsaturated double bond and more than twenty carbons, they were all shifted to the liver alone and could not be demonstrated in the extrahepatic tissues (Fig. 10). On the contrary, rabbits could tolerate the sesame oil emulsion well even when it was repeatedly given intravenously in three times larger doses than usual and without the aid of methionin. The rabbits rather gained weight after 20 weeks, and histochemically phospholipid accumulation could not be demonstrated in the liver. The paper chromatogram (Fig. 9) showed almost no difference from the normal one (Fig. 8), except for a slight increase in the amount of linoleic acid in various organs.

The metabolism of fat in vivo can be summarized schematically as shown in Fig. 17 according to the various types of fatty acids.

When the cats were starved suddenly, weight loss became marked, and subcutaneous and omental fatty tissues disappeared almost completely. When the fats in various organs were analysed by paper chromatography, the essential fatty acids such as linoleic acid, linolenic acid etc. showed practically no decrease, whereas higher saturated fatty acids, oleic acid etc. showed a marked decrease (Fig. 12).

It might be interpreted that the former plays a role as a composing factor of tissue cells i. e. the constant element; whereas the latter i. e. higher saturated fatty acids and oleic acid acts as a variable element.

From these findings we can conclude that sesame oil emulsion has the best composition of fatty acids for parenteral use. This fact has been proved and assured by our histochemical and biochemical investigations.

In other words triglycerides such as sesame oil seem to be best suited to parenteral administration of fat, since they contain no lower fatty acids, no highly unsaturated fatty acids, docosenoic acid, or eicosenoic acid etc. which for the most part are those undergoing socalled indirect oxidation; but do contain higher fatty acids and oleic acid which are used in the body as variable elements, imposing little burden on the liver, having little possiblity of forming ketosis, and undergoing for the most part direct oxidation; and also contain linoleic acid and linolenic acid which are indispensable in the composition of tissue cells as constant elements.

We can also conclude that it is desirable to use fats containing the least possible amount of fatty acids such as the lower fatty acids, highly unsaturated fatty acids, eicosenoic acid, docosenoic acid etc. for parenteral administration. Our colleague A. KISHIMOTO has affirmed that sesame oil produces an auto-oxidized substance by rancidity when the sesame oil is heated (under 90°C) or by a solar light for a long time, showing a marked increase in peroxide value and a marked decrease in the iodine value. When this auto-oxidized substance is produced, although there is no change in the amount of saturated fatty acids, essential fatty acids having unsaturated double bonds show a definite decrease. At the time of this reaction the amount of 2,4-dinitrophenylhydrazones of *p*-bromophenacylester produced is very little, and a black viscous substance which is ether insoluble is precipitated. When sesame oil is heated over 200°C polymerization takes place at the position of the double bond, and although there is no change in the amount of saturated fatty acids in the sesame oil, the amount of essential fatty acids decrease. When the auto-oxidized product enters the body it is accumulated in the tissue cells, destroying the mitochondria, and the proceeding polymerization lowers the absorption and digestion of the fat. Moreover, as this experiment shows, the rancidity or polymerization induces a decrease in the essential fatty acids, which have a much more important nutritional value than as a mere source of calories, and thus seems to deprive the fat of its nutritional value. Therefore, apart from the method of administration of the fat, it is necessary to select a fat which contains no product due to rancidity or polymerization, in other words non-spoiled fat.

The same care is to be taken in the preservation of fat, avoiding light, keeping it at low temperatures, and exposing as little surface as possible to the air. As the reaction is due to self contact, spoiled fat facilitates the reaction, therefore old oil must not be mixed with new. Furthermore since the heating of fat facilitates polymerization, inhibits digestion and absorption, and causes a decrease in the amount of the essential fatty acids, care must be taken even at the time of cooking.

V. SUMMARY AND CONCLUSION

The purpose of this biochemical experiment is to see whether or not synthesized water soluble fats are effective as solutions for parenteral administration of fats, also to see what kind of fats are the most effective as emulsions for intravenous use.

The author has synthesized water soluble alkaline salts of lower fatty acids, hydroxyl esters of higher fatty acids, hydroxylated acids, hydroxyl esters of hydroxylated acids etc., and has investigated their toxicity and nutritional value.

Paper chromatography of the fatty acids was used to analyse the composition of the fatty acids in various organs of animals after injection of various kinds of fat emulsions and at the time of starvation.

The following facts have been obtained.

1) The water soluble fatty acid salts which OMURA has mentioned cause a marked hemolysis and cannot be tolerated in clinical application.

2) When the hydrophobic higher fatty acids are combined with a hydrophilic group in order to make them water soluble, the newly synthesized water soluble

fatty acids have the surface activity of non•polar-polar compounds, and cause severe side effects such as hemolysis, and cannot be tolerated in clinical application.

3) When hydrophilic OH-radical is added to the hydrophobic fatty acids in order to lower the surface activity, the hydroxylated acids have no toxicity but have no nutritional effect as they do not enter the metabolic pathway in the body.

4) When a hydrophilic group is added to the above mentioned hydroxylated acids, although the surface activity and the toxicity decrease, they lose their nutritional value.

5) Parenchymatous organs such as liver, kidney, heart etc. contain a large amount of essential fatty acids, higher saturated fatty acids, oleic acid etc. in the postabsorptive state, but no lower fatty acids at all.

6) The lower fatty acids are shifted to nowhere but the liver. Highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. also seem to be shifted to the liver for the most part, but partially shifted to other organs also. On the contrary, higher saturated fatty acids, oleic acid, the essential fatty acids etc. seem to be shifted not only to the liver but also to various other organs, and undergo further metabolism.

7) Therefore the lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. are the ones which undergo, for the most part, socalled indirect oxidation; and administration of these fatty acids in a large quantity seems to impose a heavy burden on the liver and marked ketone body production. On the contrary, higher saturated fatty acids, oleic acid, and the essential fatty acids are the ones which in general undergo direct oxidation, so administration of fat which consists of these fatty acids alone seems to be ideal since the burden on the liver is not heavy and the ketone body production is not great.

8) When sesame oil emulsion is infused in large amounts daily for approximately twenty weeks into rabbits having a lower capacity for disposition of fat, no change is noted in the composition of the fatty acids in various organs, except for a slight increase in the amount of linoleic acid which is an essential fatty acid. This is another reason that sesame oil seems to be the ideal material for fat emulsion.

9) When the experimental animals are suddenly starved, the essential fatty acids such as linoleic acid, linolenic acid etc. show no change in amount, but the higher saturated fatty acids and oleic acid show a marked decrease in amount. This leads us to assume that the former has a meaning as a constant element and the latter as a variable element.

10) When rats are fed non-fat diet for a long period of time, higher saturated fatty acids and oleic acid show no decrease in amount as they are synthesized in the body, but the essential fatty acids such as linoleic acid show a definite decrease in amount which leads us to assume that they are not synthesized in the body.

11) When sesame oil is heated (under 90°C) for a long time, it becomes rancid and produces auto-oxidized substances, and the peroxide value rises. In this process, even though saturated fatty acids show no change in amount, linoleic

acid having double bonds show a definite decrease in amount, lowering the nutritional value of sesame oil as a fat. In the process of polymerization due to heating (over 200°C) there is also a decrease in the amount of the essential fatty acids. Therefore care has to be taken in the preservation and cooking of the oil.

The above findings indicate that at present fat cannot be used in parenteral injections as a water soluble substance but must be used in the form of an emulsion intravenously.

Material for emulsions must be chosen from among the fats which do not contain lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc., which undergo indirect oxidation; but which contain higher saturated fatty acids and oleic acid which undergo direct oxidation and also the essential fatty acids which are indispensable for the composition and function of the tissue cells.

This has lead us to believe that sesame oil is the ideal substance, and one must select refined sesame oil with the lowest peroxide value.

I wish to express my sincere gratitude to Dr. Y. Hikasa for his helpful suggestion and kind guidance in the course of the work. Grateful thanks are also due to Dr. H. Kuyama for the kind guidance in the organic chemical fields, and Dr. M. Noda and Dr. O. Hirayama for their kind suggestion about paper chromatography.

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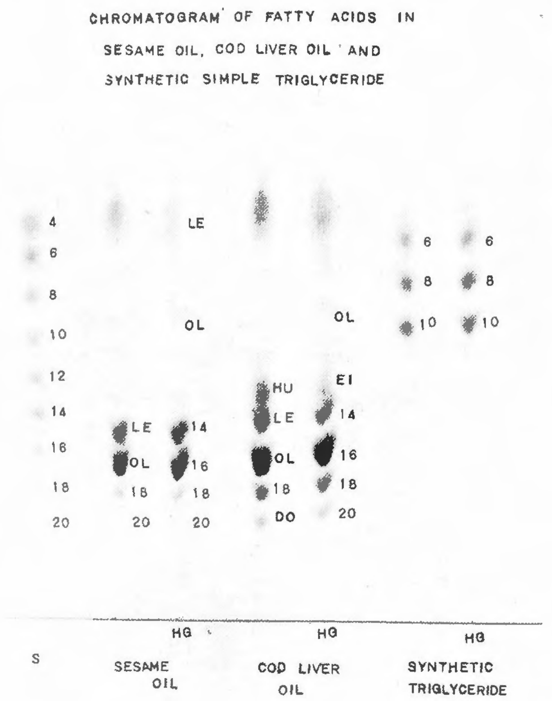


Fig. 3 Chromatogram of fatty acids in sesame oil, cod liver oil and synthetic triglycerides

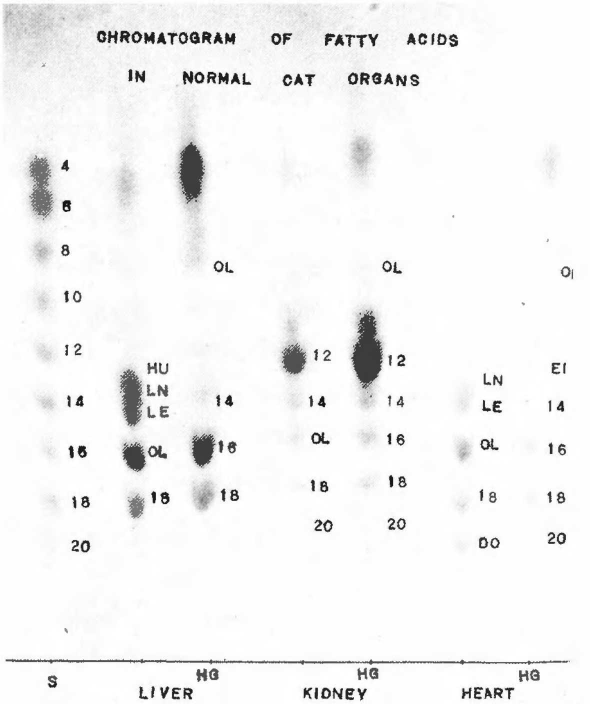


Fig. 4 Chromatogram of fatty acids in normal cat organs

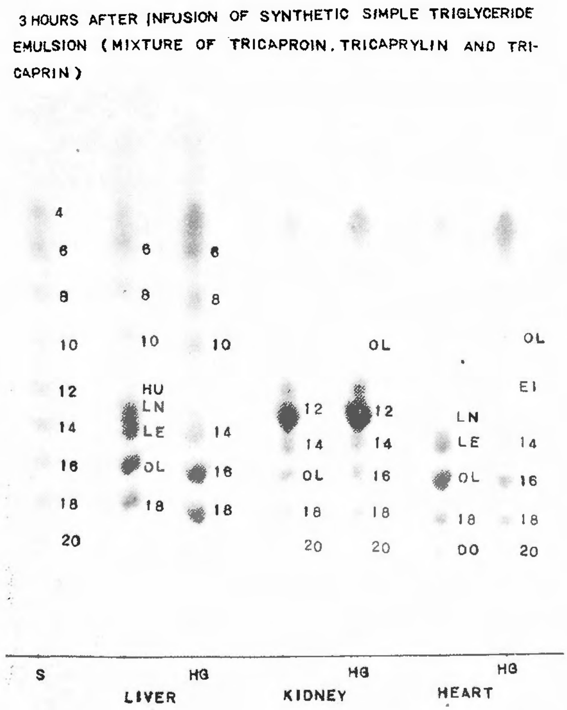


Fig. 5 3 hours after infusion of synthetic simple triglyceride emulsion (mixture of tricaproin., tricaprylin and tricaprin)

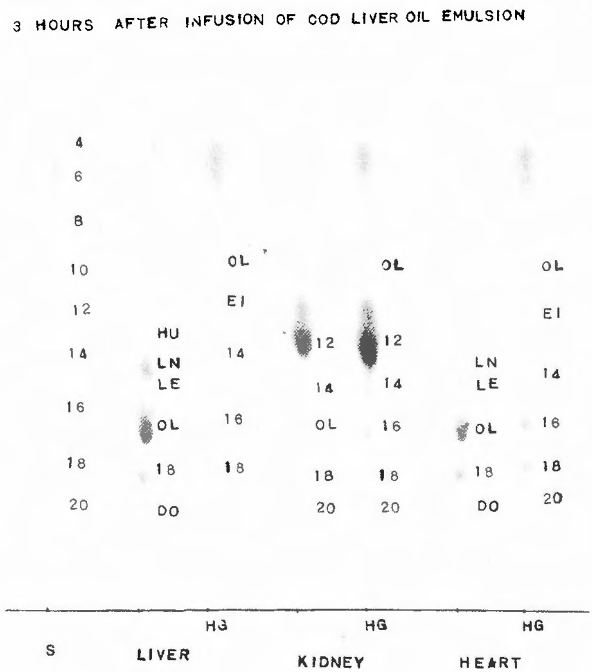


Fig. 6 3 hours after infusion of cod liver oil emulsion

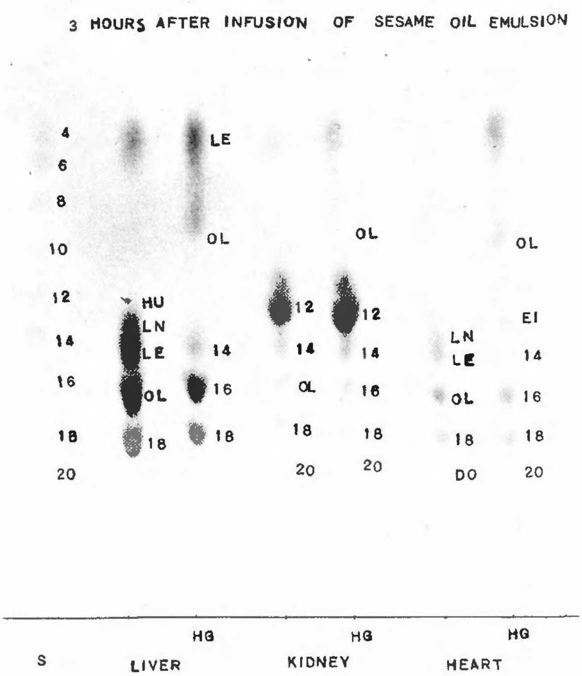


Fig. 7　3 hours after infusion of sesame oil emulsion

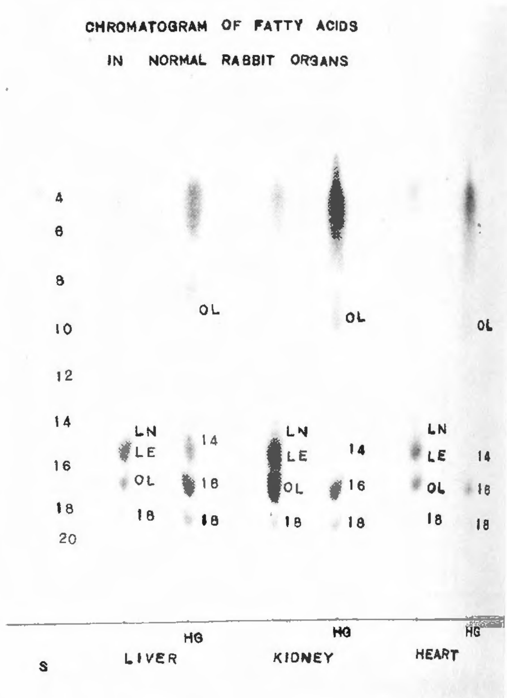


Fig. 8　Chromatogram of fatty acids in normal rabbit organs

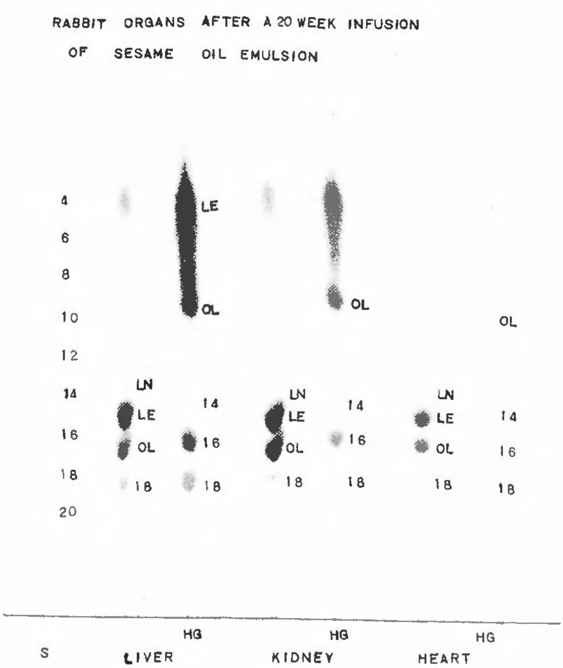


Fig. 9　Rabbit organs after a 20 week infusion of sesame oil emulsion

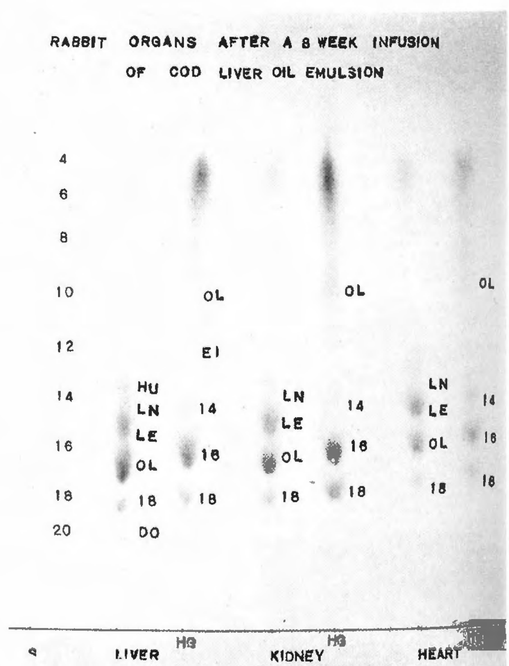


Fig. 10　Rabbit organs after a 8 week infusion of cod liver oil emulsion

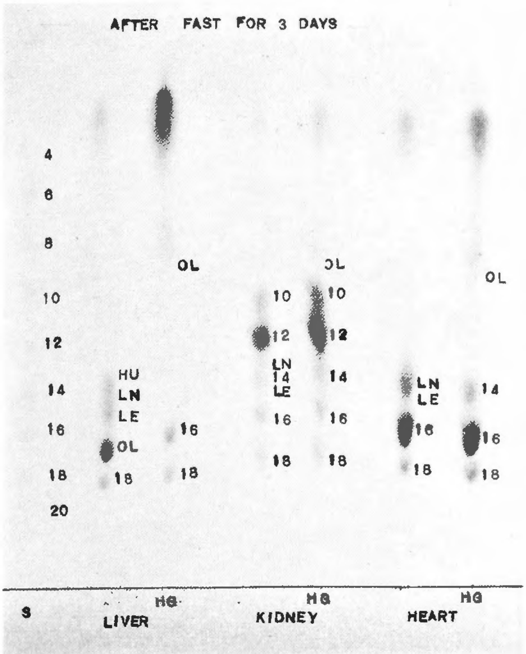


Fig. 11 After fast for 3 days

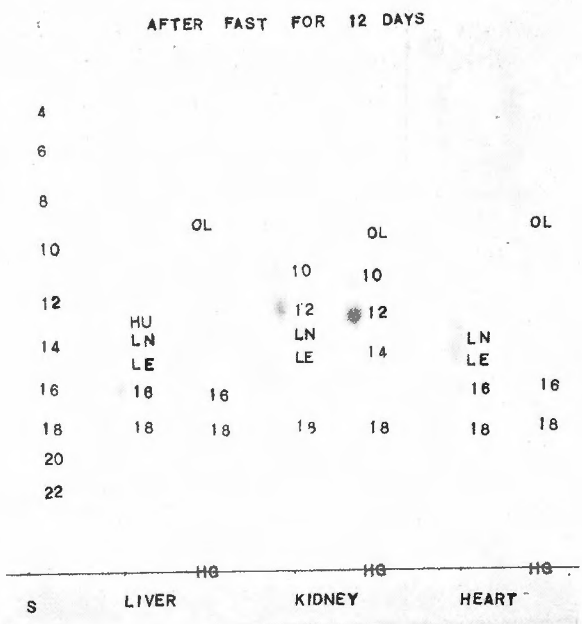


Fig. 12 After fast for 12 days

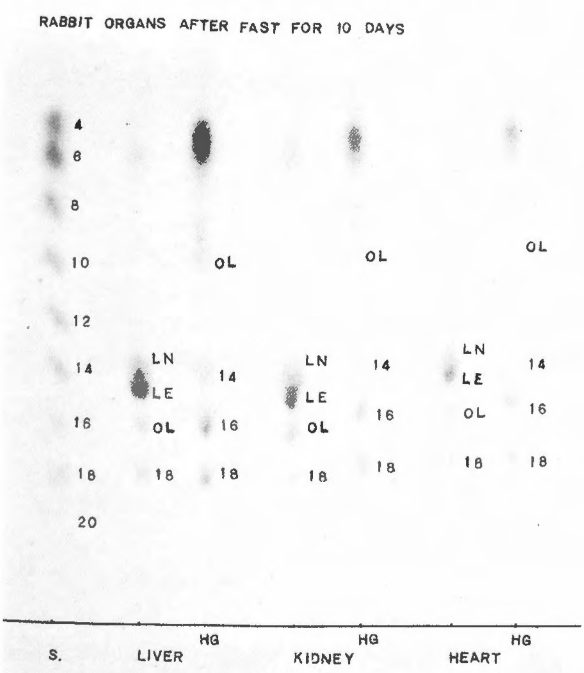


Fig. 13 Rabbit organs after fast for 10 days

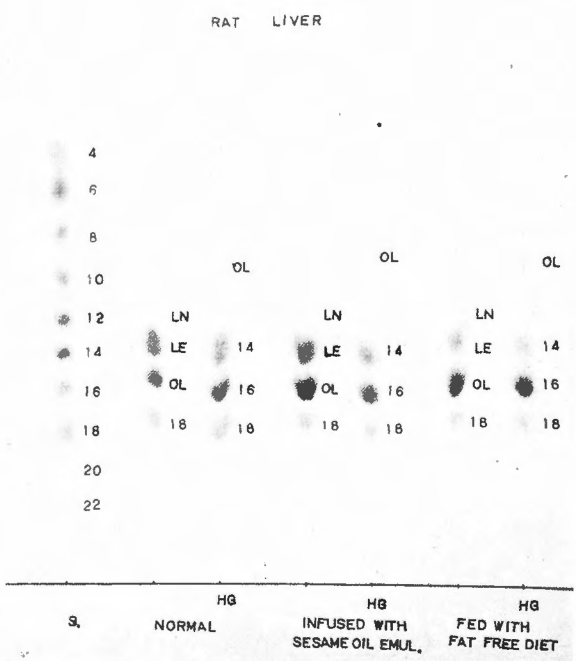


Fig. 14 Rat liver

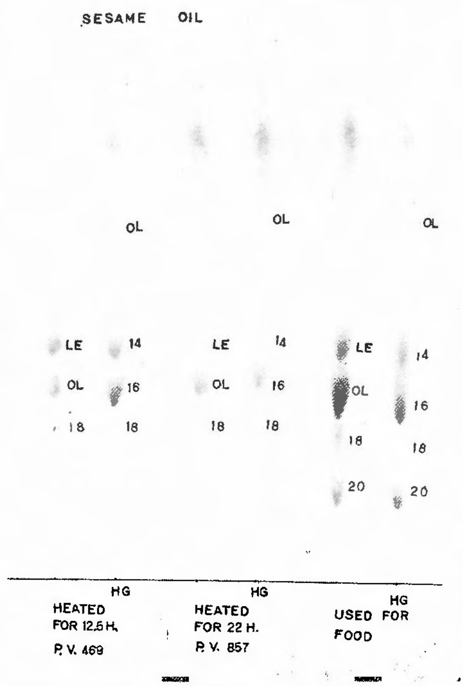


Fig. 15 Sesame oil heated under 90℃

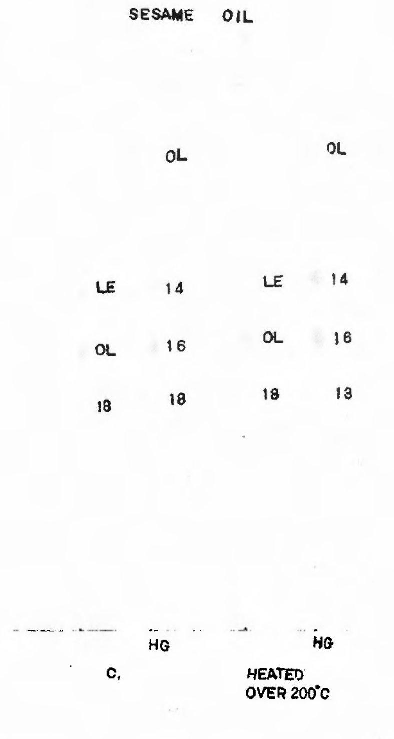
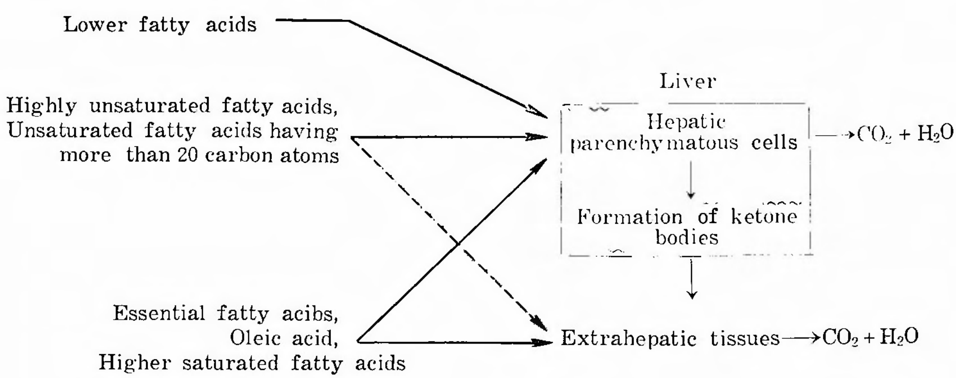


Fig. 16 Sesame oil heated over 200℃

Fig. 17. Metabolic process of fat in vivo



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和 文 抄 録

各種脂肪の栄養学的効果についての実験的吟味

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著者は，合成水溶性脂肪が，脂肪輸液として有効的なものであるかどうか，或は又，脂肪乳剤を経静脈的に輸入する場合には，如何なる組成を有する脂肪が生体に最も有効的であるかを，実験に亘した．

即ち，低級脂肪酸の水溶性アルカリ塩，高級脂肪酸の水酸基エステル，オキシ脂肪酸，オキシ脂肪酸の水酸基エステル等を合成して，その毒性及び栄養効果について検討を加え，更に，脂肪酸ペーパー・クロマトグラフィーを応用し，脂肪処理能力の旺盛な肉食動物である猫，及び脂肪処理能力の悪い家兎に，各種の脂肪乳剤を注入した場合，また或いは飢餓状態においた場合の各種臓器の脂肪酸組成について分析した結果，次の結論に達した．

1) 大村の提唱した綜合水溶性脂肪酸塩は溶血現象が著明で臨床的使用には到底耐え得ない．

2) 不溶性の高級脂肪酸を水溶性にする目的で親水基を結合させると，新たに合成された水溶性脂肪はNon-polar-polar compounds の表面活性剤として作用して表面活性作用を呈し，溶血現象等の重篤な副作用を起す主因となり，使用に耐えない．

3) 表面活性作用を低下させる為に，疎水基の脂肪酸に親水基OHを導入した所謂オキシ脂肪酸は，毒性を有しないが生体代謝過程には利用されず，栄養効果を有しない．

4) 上記オキシ脂肪酸に，親水基を結合させると，表面活性作用は低下し，毒性は減ずるが栄養効果を有しない．

5) Postabsorptive state に於ては実質臓器である肝臓，腎臓，心筋等には，不可欠脂肪酸，高級飽和脂肪酸及びオレイン酸を多く含み，低級脂肪酸は全く認められない．

6) 低級脂肪酸は専ら肝臓へのみ移行し，その他の臓器へは全く移行しない．又高度不飽和脂肪酸，ドコセン酸，エイコセン酸等も主として肝臓へ移行するがなおその幾許かは肝外組織中へも移行し得るものと考えられる．それに反して，一般高級飽和脂肪酸，オレイン酸，不可欠脂肪酸等は肝臓のみならず，肝外組織中にも活潑に移行し，その後の処理を受けているものということが出来る．

7) 従つて低級脂肪酸，高度不飽和脂肪酸，ドコセン酸，エイコセン酸等は所謂間接的酸化型式を営む比率の大なる脂肪酸であり，このような脂肪の多量摂取は肝臓に対する負担も大で，且つケトージス発生の程度も著しいものと考えられる．これに反して，高級飽和脂肪酸，オレイン酸，不可欠脂肪酸は直接的酸化型式を営む比率の大なる脂肪酸であるということが出来て，これら脂酸のみからなる脂肪の摂取時には，肝臓の負担を少なくし，ケトージス発生の怖れも極めて少なく，生体にとつて最も合理的な脂肪酸であるというこが出来る．

8) ゴマ油乳剤を，脂肪処理能力の弱い家兎に約20週間にわたり，連日大量注入しても，各種臓器とも，不可欠脂肪酸であるリノール酸の増量を認める程度で，その他の脂肪酸組成には全く変化を認めない．従つてこのような意味からもゴマ油は脂肪乳剤の原料として適当なものと思われる．

9) 試獣を急性飢餓状態におくと，リノール酸，リノレイン酸等の不可欠脂肪酸には殆んど減量する傾向を認めないにも拘らず，一般高級飽和脂肪酸及びオレイン酸は著しい減量を認める．即ち前者は constant element として，後者は variable element としての意義を有することが推測される．

10) ラッテを無脂肪食で比較的長期間飼育した場合一般高級飽和脂肪酸及びオレイン酸は体内で合成され

るため，減量を認めないが，リノール酸等のような不可欠脂肪酸は明らかに減量し，生体内で全く合成し得られない事実が推測される．

11) ゴマ油を長時間にわたり，通気加熱するか，或いは又太陽灯照射を行うと自働酸化反応を惹起し，自働酸化生成物を生じ，Peroxide value は上昇する．このようになると，ゴマ油が含有する飽和脂肪酸量には変化を認めないが，二重結合を有するリノール酸等の不欠可脂肪酸は明らかに減少する為，脂肪としての栄養価は著しく低下する．且つ又，過熱による重合反応に際しても不可欠脂肪酸の減量を招く．従つて油脂の保存調理にも充分な注意を要する．

以上の事実から，現在の段階に於ては，脂肪輸液としての脂肪は，それを水溶性にして利用することは不可能で，脂肪を乳化態として経静脈的に注入する他はない．

而も，以上の事実から，間接的酸化型式をとる低級脂肪酸，高度不飽和脂肪酸，ドコセン酸，エイコセン酸等を全く有しないで，直接的酸化型式をとる一般高級飽和脂肪酸，オレイン酸，及び生体の組織細胞の構成並びにその機能遂行に不可欠な所謂不可欠脂肪酸をも充分に含有した脂肪をその原料として選ぶべきであり，そのような意味に於て，ゴマ油は最も適当なものであると思われる．

而してゴマ油に於ても，Peroxide value の低く，且つ精製されたゴマ油を選ぶことが最も望ましいのである．